JEMTAC
Journal of Emergency Medicine Trauma & Acute Care
A PEER REVIEWED JOURNAL

OPEN ACCESS

Conference Paper

Nano vesicular topical drug delivery system: Types, structural components, preparation techniques, and characterizations

Milad Jawad Hasan[1], Nawal Ayash Rajab[2,*]

[1]National Center for Drugs Control & Research, Ministry of Health, Baghdad, Iraq
[2]Department of Pharmaceutics, College of Pharmacy, University of Baghdad, Iraq

*Email: dr.nawalayash@copharm.uobaghdad.edu.iq

ABSTRACT

Nano vesicles, one of several existing nanoscale drug delivery systems, offer extremely promising new ways to develop treatments for degenerative diseases, cancer, and inflammation. The primary goals in developing nanocarriers are to control particle size, surface characteristics, and drug release to achieve specific objectives. Therefore, it is crucial to accurately characterize nanocarriers to effectively regulate their intended behavior both in clinical settings and in the body. Nanocarriers are characterized by their size, structure, and charged state, which are determined by sophisticated microscopic methods such as scanning electron microscopy, transmission electron microscopy, and atomic force microscopy. Electron microscopy is used to evaluate the surface morphology and size of particles, while dynamic light scattering and photon correlation spectroscopy are used to estimate particle size and size distribution. Colloidal stability is determined by zeta potential, which is an indirect measure of surface charge, and differential scanning calorimetry is used to characterize particles and drug interactions.

Keywords: drug delivery systems, nano vesicles, cubosomes, invasomes, ufasomes

https://doi.org/10.5339/jemtac.2024.absc.16

Submitted: 19 February 2024
Accepted: 16 May 2024
Published: 23 October 2024

QSCIENCE
HAMAD BIN KHALIFA UNIVERSITY PRESS

Cite this article as: Hasan MJ, Rajab NA. Nano vesicular topical drug delivery system: Types, structural components, preparation techniques, and characterizations. *Journal of Emergency Medicine, Trauma & Acute Care*. **2024(6):16** https://doi.org/10.5339/jemtac.2024.absc.16

INTRODUCTION

Innovative drug delivery techniques have been made possible by the ongoing development of materials and technologies brought about by the combined efforts of several fields, including materials science, chemistry, bioengineering, physics, medicine, and materials science. Researchers and clinicians worldwide are continually developing and using novel pharmacologically active chemicals and Nanoparticles to create safer, more affordable, and customized treatment alternatives.

Drug delivery efficiency has recently been demonstrated through the use of nanoparticles. In general, a material is considered a nanomaterial if at least one of its dimensions is between 1 and 100 nm.[1] Nano-drug delivery technologies can be used to increase the stability and solubility of pharmaceuticals by prolonging their blood circulation time and improving their delivery effectiveness due to their morphological, optical, mechanical, and electrical properties. Recently, studies have been carried out on nanoscale metallic, polymeric, organic, and inorganic materials, such as dendrimers, nanotubes, micelles, and quantum dots, as drug delivery carriers (DDC).[2] The ability of nano medicines to encompass drugs or to combine medicinal substances with the nanostructures and carry them to specific tissues in a more precise and controlled manner has drawn attention in recent years. Research has described the application of nanoscale materials – such as nano robots, nano sensors, and actuator materials – in living cells to achieve sensory, delivery, or diagnostic purposes.[3] Drug delivery involves the use of various types of nano carriers. Modifying the solubility of hydrophobic materials, achieving controlled or sustained release, enhancing drug stability, and ultimately targeted therapy at the site of action that increases efficacy and minimizes side effects are only a few of the many advantages it offers over conventional drug delivery systems (DDS).[4]

Nano vesicles, one of several existing nanoscale DDCs, offer extremely promising new ways to develop treatments for degenerative diseases, cancer, and inflammation.[1] A drug formula can be applied topically to the skin for systemic absorption using a technique known as transdermal drug delivery. The method, which is completely painless, begins with the drug penetrating the stratum corneum, the outermost layer of the skin, and moving down to the dermis and epidermis, where it is available for systemic absorption.[8] Topical drug delivery offers various advantages over other routes of administration, including lower side effects and patient compliance, since it avoids the first-pass impact and the pre systemic metabolism of drugs in the liver. Additionally, patients who are unable to swallow their pills could use this alternative route. The drug delivery process ends when the formula is withdrawn from the skin surface. However, the fundamental problem with these systems is the transport of substances via the small pores of the skin, which are between 20 and 40 nm in size and act as a barrier.[5,6]

Lipid-based vesicular systems such as liposomes and conventional niosomes can effectively accommodate both hydrophilic and lipophilic drugs. The main obstacles of these systems are payload leakage, and difficulty in scaling up.[7] Recent developments of “soft” nano carriers provide several benefits compared to traditional vesicular-type systems. This study focuses on the modern lipid-based nano vesicular structures used in a variety of clinical and biological settings. We discuss nano vesicular carriers such as cubosomes, invasomes, and ufasomes which may be less well known but are increasingly being investigated for drug delivery applications.

CUBOSOMES

Cubosomes are hydrated surfactant structures capable of forming a bicontinuous liquid cubic crystal phase while maintaining thermodynamic stability through self-association. These systems are binary mixtures that are both viscous and isotropic. The incorporation of adjuvants is increased due to the highly twisted structure of the lipid bilayer and the large surface area (approximately 400 m^2/g) of cubosomes.[8] Cubosomes consist of two internal water channels and three-dimensional honeycomb-like structures composed of curved, bicontinuous lipid bilayers (Figure 1). Many bioactive molecules such as peptides, proteins, and chemical drugs can use these channels.[8]

The definition of both open and closed cubosome shapes can be determined using differential geometry techniques. The open cubosome has aqueous channels that provide a link to the external surrounding area. While the closed cubosome has a single water channel that is open to the outside and another isolated channel. Cubosomes have cubic symmetry, similar to their bulk parent phase, and can be categorized as gyroid, primitive, or diamond.[9]

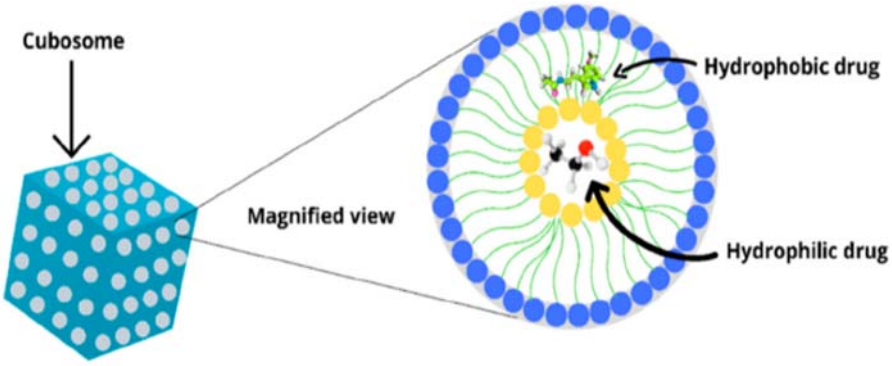

Figure 1. Structure of a cubosome lipid vesicular system.[9]

Advantages of cubosomes

Cubosomes have reduced viscosity compared to the cubic phase gel and the cubic phase precursor. These nanoparticles exhibit exceptional stability when stored at ambient temperature, possess greater resistance to high temperatures, and exhibit low drug leakage compared to liposomes.[10] Cubosomes can be used to load drugs that are hydrophilic, lipophilic, or amphiphilic.[8]

1. It is cost-effective.
2. It has no toxicity and exhibits biocompatibility.
3. The method of preparation is simple.
4. It has exceptional bioadhesive characteristics.
5. It shows an increase in skin permeation.
6. They exhibit thermodynamic stability over extended periods of time.
7. They offer targeted delivery and regulated release of bioactive substances.
8. The increased drug loading is due to the large internal surface area and cubic crystal formations.
9. They have reduced viscosity and can maintain their presence in water at any level of dilution, unlike other liquid crystalline drug delivery methods such as liposomes and hexosomes.[9]

Disadvantages of cubosomes[9]

1. The high water content in cubosomes results in reduced entrapment of water-soluble drugs.
2. Large-scale manufacturing can sometimes be challenging due to high viscosity.
3. There is a potential for particle aggregation in the absence of agitation over an extended period of time.

Structural Components

Amphiphilic lipids

Phytantriol and monoolein are the two lipids most commonly used to produce cubosomes. Both lipids are approved for use in vivo and are biocompatible.[11] Glyceryl monooleate (GMO), also known as monoolein, is an amphiphilic lipid used in the preparation of cubosomes (Figure 2(A)). The chemical structure of GMO reveals its amphiphilic nature, which has both hydrophilic and hydrophobic characteristics. This is due to the presence of hydroxyl groups in the head part, which facilitate the formation of hydrogen bonds with water, and the presence of hydrocarbon chains in the tail part.[12] PHYT (3,7,11,15-tetramethyl-1,2,3-hexadecanetriol), also known as phytantriol, is used as a component of cosmetics and is considered a perfect replacement for monoolein in the production of cubosomes (Figure 2(B)). This is because lipid-based substances such as GMO are easily broken down by esterases. However, phytanyl backbone of PHYT could provide greater structural stability.[12]

Stabilizers

Stabilizers are added to the particles dispersed in water to prevent them from re-coalescence into the cube structure of the parent aggregate. Pluronic F127 is an example of such a stabilizer (Figure 2(C)).[12] Poloxamer-407 (P407 or Pluronic F127 or PF127) is a triblock polymer composed of polyethylene oxide, polypropylene oxide, and polyethylene oxide (PEO – PPO – PEO) copolymer.[12] In addition, the PEO

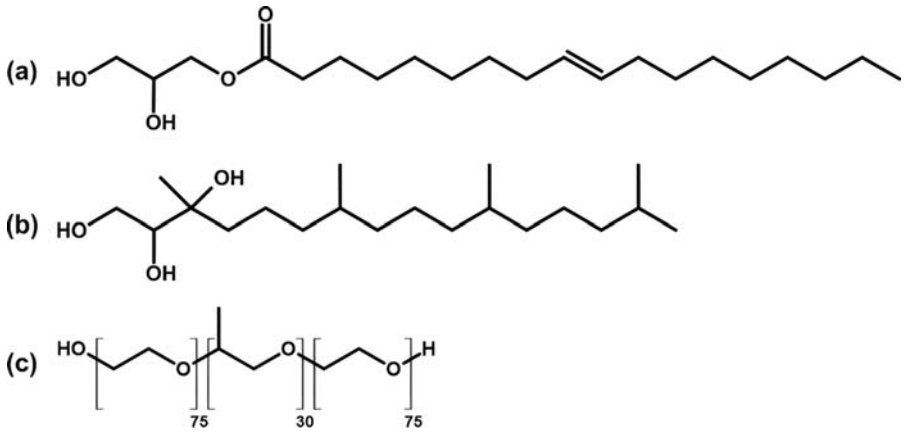

Figure 2. Structure of (A) glyceryl monooleate, (B) phytantriol, and (C) poloxamer-407 (P407 or Pluronic F127).[12]

component of the polymer is responsible for its hydrophilic characteristics, while the PPO component explains its hydrophobic characteristics. The stabilizing effects of P407 account for the ability of the hydrophobic PPO block to adsorb or be functionalized on the particle surface. Furthermore, the hydrophilic PEO component provides steric shielding by extending into the nearby aquatic environment. Tween 80, PEO – PPO – PEO, and PEGylated polymers are other examples of stabilizers.[13]

Preparation of cubosomes

Cubosomes can be prepared using three different methods, which are described below.

Top-down technique

Cubosomes nanoparticles are extensively used in research. The process first involves a large quantity of bulk cubic phase, which is then dispersed into cubosome nanoparticles through high-energy processing. The bulk cubic phase is a solid, gel-like substance consisting of water-swollen polymer chains that are cross-linked. In contrast, cubic phases have a structure that is characteristic of liquid crystals. The cubic phases have a stress level at which deformation begins to occur (yield stress) that increases with increasing amounts of bilayers producing surfactants and oils. According to most previous studies, when comparing the dispersion resulting from the use of ultrasound and high pressure homogenization techniques, it indicates the formation of delicate dispersions composed of vesicles and cubosomes. The proportions of these particle types vary over time. Coarse micro-scale cubosomes have the same D-surface structure as the bulk cubic phase from which they originate. However, after homogenization, the P-surface becomes dominant due to the presence of additional polymers.[14]

Bottom-up technique

Cubosomes can develop or crystallize from precursors. In the bottom-up strategy, nanoscale structural components are first formed and then assembled to produce the final material. This recently discovered approach enables the production of cubosomes by allowing them to form and crystallize from precursors at the molecular level. The crucial element of this approach is the hydrotrope, which has the ability to break down water-insoluble lipids into liquid precursors. This strategy uses dilution to produce cubosomes with lower energy requirements compared to the top-down approach.[13]

Thin-film methods

The lipid is dissolved in a predetermined amount of an organic solvent, either with or without the addition of a drug. The organic solvent is evaporated using a rotary evaporator with an oil pump under reduced pressure, at a certain temperature and speed, until a thin layer is formed. Subsequently, a solution of either aqueous phosphate buffer or phosphate buffer saline (PBS) containing P407 as a stabilizer is introduced into the lipid film in its dry state. The mixture is then subjected to sonication using a bath sonicator.[15]

UFASOMES

Ufasomes are a mixture of unsaturated fatty acid vesicles formed by combining fatty acid and ionic surfactant (soap) with cholesterol (Figure 3). They serve as an effective drug delivery vehicle for dermal administration. The stratum corneum, the outermost layer of skin, acts as the primary resistance to drug penetration. The use of ufasomes as a DDS can effectively solve this issue because ufasomes are composed of a lipid membrane that can easily adhere to the skin.[16,17]

The formation of unsaturated fatty acid vesicles (ufasomes) has been observed to occur within a specific pH range of 7–9. In this range, the fatty acid carboxylic acid groups, which can exist in both ionized and unionized forms, organize themselves into bilayer vesicles.[16] Ufasomes exhibit superior stability and cost-effectiveness compared to liposomes.[17] The degree of stability of ufasomes depends on the choice of fatty acids, the inclusion of cholesterol, the pH level, the selected buffer, the presence of divalent cations, and the amount of lipoxygenase used.[22]

Advantages of ufasomes[18]

1. Ufasomes enhance the residence time of a drug in the bloodstream while reducing its toxicity.
2. They are more cost-effective than liposomes and are noisome due to the widespread availability of fatty acids.
3. They penetrate quickly when applied topically.
4. Due to their targeted delivery, they have the potential for selective absorption.
5. Their entrapment ability is satisfactory.
6. They prolong the elimination of rapidly metabolized compounds and function as a sustained release mechanism.
7. Hydrophilic or lipophilic drugs can be integrated into ufasomes.
8. They enhance bioavailability, especially for drugs with low solubility.

Disadvantages of ufasomes[19]

1. They may induce atherosclerosis.
2. The rapid oxidation of fat-based drugs poses a stability issue.
3. Certain oxidation byproducts can be very hazardous to biological systems.

Components of ufasomes

Fatty acid surface

Film-based analysis and information investigations indicate that the use of fatty acids with carbon chain lengths ranging from 12 to 22 is suitable for the development of stable ufasomes. Studies were limited due to the superior performance of C-18 fatty acids in the initial experiments. Ufasomes can only be prepared using membranes composed exclusively of unsaturated oleic fatty acid (cis-9-octadecenoic acid) and unsaturated linoleic fatty acid (cis, cis-9, 12-octadecadienoic acid). Oleic acid maintains its purity for at least six weeks, but peroxides begin to form significantly after two to three weeks, as indicated by stability tests.[20]

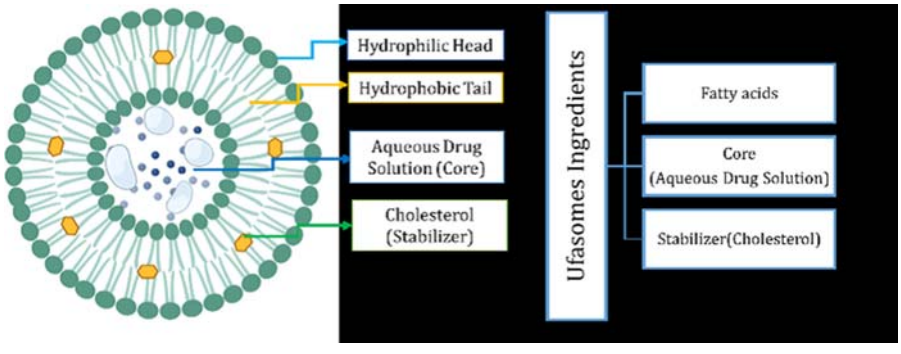

Figure 3. Structure of ufasomes lipid vesicular system.[1]

Cholesterol

Cholesterol contributes to the stability of vesicle membranes by increasing their rigidity and reducing drug leakage.[21] Cholesterol serves to occupy the spaces resulting from the improper arrangement of other lipid molecules. The presence of larger amounts of cholesterol leads to a significant decrease in the ability of the vesicle to retain solutes. Furthermore, the impermeability of the membrane does not increase with any quantity of cholesterol.[22]

Methods of preparation[23]

Thin-film hydration method

In this approach, vesicle formation occurs within a narrow pH range. Fatty acids are mixed with a natural solvent in a flask with a circular rim. This method requires a significant focus on fatty acids. The liquid evaporates before the natural solvent completely evaporates. Ultimately, by using a buffer with a suitable pH, a thin layer of fatty acids is formed and hydrated.

Alcohol addition method

In this technique, lipid vesicles are formed by adding an alcohol that has an identical chain length to the fatty acid. The approach ensures the stability of fatty acid vesicles throughout a broad pH spectrum, which confers a notable benefit. An important advantage of this method is that the fatty acid vesicles are stable over a broad pH spectrum.

Autopoetic process method

Fatty acid vesicles form due to the unpredictable change in pH that occurs when an aqueous fatty acid solution is introduced into a water-buffered solution. Vesicles can form when 50% of the carboxylic acids in fatty acids are ionized. In contrast to the aqueous component, the hydrocarbon chain forms a bilayer arrangement that minimizes interaction with water.

INVASOMES

Invasomes are a type of vesicles that contain terpenes and are used to improve skin penetration, surpassing the effectiveness of traditional liposomes. These liposomal vesicles are characterized by their softness, elasticity, and high fluidity of the membrane. They include terpene and ethanol, as shown in Figure 4, which serve as penetration enhancers, thereby making them distinct. This unique feature leads to higher drug efficacy, enhanced patient compliance, and greater comfort.[24]

Advantages of invasomes[24]

1. This approach is a drug delivery method that does not require any invasive procedures.
2. It increases the speed at which active ingredients can penetrate the skin.
3. It is possible to deliver hydrophilic and lipophilic drugs to specific targets.
4. It increases patient compliance due to the availability of the drug in a semi-solid formulation such as cream or gel.
5. The invasome formulation contains harmless excipients.

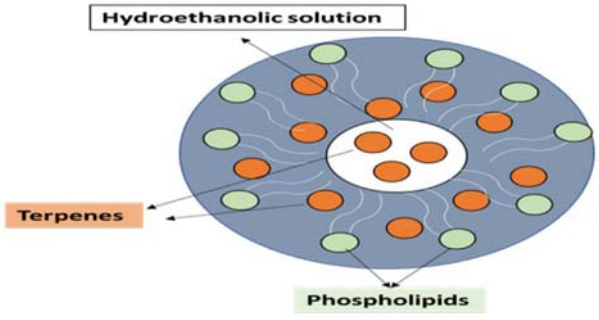

Figure 4. Structure of invasome.[28]

Disadvantages of invasomes[24]

1. There is a possibility of leakage and fusion of encapsulated active substances.
2. Manufacturing involves significant costs.
3. The presence of phospholipids in invasomes can lead to oxidation or hydrolysis, which can affect the stability of the vesicles.

Components of invasomes

1. Invasomes are innovative elastic lipid vesicles consisting of phosphatidylcholine, ethanol, and one or a combination of terpenes.[25] Terpenes are hydrocarbon compounds that serve as a basic component of essential oils in many plants. Furthermore, the presence of terpenes can lead to the formation of deformable vesicles. Several studies have extensively shown that terpenes improve percutaneous penetration. Their ability to enhance permeation is achieved by disrupting the lipids of the stratum corneum, interacting with intracellular proteins, and improving the distribution of the drug into the stratum corneum. Ethanol increases the vesicle capacity to penetrate the stratum corneum. Furthermore, ethanol induces a net negative surface charge and inhibits vesicle aggregation through electrostatic repulsion.[25] Ethanol is an effective substance for enhancing penetration, while terpenes have been shown the ability to promote the penetration of various drugs by breaking down the tightly packed lipids in the stratum corneum.[26]

Method for preparing invasomes

Mechanical dispersion technique [27]

Drugs are dissolved together with terpene or combinations of terpenes in a solution of ethanol and phospholipids. The resulting mixture is stirred vigorously with a vortex mixer for 5 minutes, and then subjected to ultrasonic waves for 5 minutes to obtain a transparent solution. A PBS solution with a pH of 7.4 is added to the solution using a syringe while continuously vortexing. The vortexing process is extended for another 5 minutes to obtain the final preparation of invasomes.

Film hydration technique[28]

In addition, the conventional film method can be used to prepare invasomes. The solvent chloroform with a volume-to-volume ratio of 2:1 is used to dissolve phospholipids in ethanol. The rotary flash evaporator is used to gradually decrease the pressure from 500 to 1 mbar at a temperature of 50°C in order to dry this mixture into a thin layer. The film is subjected to a vacuum at ambient temperature (1 mbar) for two hours before being purged with nitrogen. In order to produce invasomes from the film, we can either add a mixture of PBS (pH 7.4), ethanol, and terpene, or simply add a single terpene after the film has been cooled to room temperature and hydrated for 30 minutes. To determine the size of the resulting vesicles, polycarbonate membranes with different pore sizes are repeatedly extruded using a vortex and ultra sonicator.

CHARACTERIZATION OF NANOVESICULAR CARRIERS

The characterization of nano vesicular carrier formulations involves the evaluation of numerous physicochemical and biological properties, as well as their stability and effectiveness in drug entrapment (Table 1).

Table 1. Characterization of nanovesicular carriers with their quality control assays.[19]

Physical characterization	
Vesicle shape and surface morphology	TEM (Transmission Electron Microscopy) and SEM (Scanning Electron Microscopy)
Vesicle size and size distribution	Dynamic light scattering, TEM
Electrical surface potential and surface pH	Zeta potential measurement and pH sensitive probes
Lamellarity	P31 NMR (Phosphorus-31 Nuclear Magnetic Resonance Spectroscopy)
Drug release	Diffuse cell/dialysis
Drug concentration	Drug concentration
pH	pH meter

The physical parameters include the assessment of vesicle characteristics such as shape, surface morphology, average size and size distribution, surface electrical potential and pH, surface charge, lamellarity, percentage of free drug and drug encapsulation, as well as their release and phase behavior. The consistent size distribution of nano vesicular carrier compositions over a certain period reflects their physical stability. The occurrence of drug leakage from the vesicles and/or the aggregation or fusion of vesicles to produce larger particles indicates the presence of physical instability. The physical parameters analyzed are shown in Table 2 along with the corresponding analytical techniques.[29]

Nanoparticle morphology identified by microscopy techniques

Electron microscopy techniques are widely used in drug nanotechnology to investigate the physical properties and size of nanoparticles. However, the choice of microscopy method and its suitability for the specific particles under study can greatly influence the outcome. The dimensions and morphology of the nanoparticles play a pivotal role in therapeutic applications.[30]

Transmission electron microscopy

Transmission Electron Microscopy (TEM) is widely used to analyze the ultrastructure of nanoparticles, particularly their shape, size, and internal composition. Three approaches, namely negative staining, freeze-fracture, and plunge freezing or cryo-TEM, can be used to characterize nanovesicular carriers using this technology.[30]

Nasr et al. developed cubosome nanoparticles as a delivery system for the corneal permeation of gatifloxacin (GTX) using a clinical strain of methicillin-resistant *Staphylococcus aureus*. The researchers examined the morphology of the developed cubosomes and found that the GTX-loaded cubosomes were nearly spherical, polyangular, non-aggregated particles with homogeneous and narrow size distribution.[31]

Scanning electron microscopy

The literature documents many methods for identifying the morphologies of closed bilayer structures such as invasomes. One such method is scanning electron microscopy (SEM), which uses an electron beam to produce an enlarged image. Therefore, a greater amount of detailed information about the shape, structure, and composition of vesicles can be obtained.[32] Prasanthi et al. developed invasomes containing finasteride for transdermal administration using the iontophoresis method. The researchers assessed the morphology of the invasomes they developed using SEM and observed that the vesicles had a spherical shape and a unilamellar configuration.[33]

Atomic force microscopy

The application of atomic force microscopy (AFM), a newly discovered microscopic method, is used to investigate the shape, size, and stability of nanovesicular carriers. AFM, a type of scanning probe microscope, has the exceptional capability to visualize microscopic nanovesicles in their natural environment without sample modification. With a dimensional resolution of approximately 0.1 nm, AFM offers a unique opportunity for detailed observation. The outcome is a meticulously detailed three-dimensional representation of the surface of the vesicle under study, with a particularly high level of clarity and precision.

The method allows the observation of nanovesicles without modifying their original structure, as long as the necessary surface immobilization does not have a negative impact on the material and the force exerted by the probe does not damage the vesicles. AFM analysis is a fast, potent, and harmless technology. It can provide data on the structure, dimensions, and potential aggregation of nanovesicular carriers during their storage.[34]

Nasr et al. developed cubosomes loaded with gliclazide. The AFM image of gliclazide-loaded cubosomal nanoparticles showed that the nanoparticles were dispersed as individual, well-defined polyangular particles with a height of approximately 52.55 nm. Furthermore, the cubosomal nanoparticles had a nanoscale size that corresponded to the observed particle size values.[35]

Nanoparticle surface charge (zeta potential)

The surface potential of nanoparticles is an essential physicochemical characteristic because it influences the degree of intra-particle interactions, the adsorption of counter-ions, and therefore particle stability. This characteristic is most typically defined as zeta potential, which reflects the charge

profile of a bare particle associated with a diffusive layer, and can be calculated from the electrophoretic mobility of particles determined using phase analysis light scattering.[36]

Particle size and poly dispersibility Index

A fundamental feature that distinguishes nanoparticles from other DDS and bulk powders is their size. The dimension of the particles affects the release of the encapsulated substances. Smaller particles have a larger surface area. As a result, most of the trapped substance is exposed to the release medium, leading to a faster release of the drug. In contrast, therapeutic agents slowly diffuse from the inner layer of the macroparticles.[37]

Encapsulation efficiency

Encapsulation efficiency (EE) is assessed using an ultracentrifuge. The drug was extracted from the vesicles by centrifugation at 15,000 rpm and 4̊C for 15 minutes in two cycles, resulting in the separation of the drug-containing vesicles from the unentrapped drug. The drug-loaded vesicles sediment to the bottom. The supernatant containing the unbound drugs is then diluted and examined with a UV–Visible spectrophotometer.[38]

The percentage of EE is calculated using the formula:

$$\mathbf{EE} = \frac{\text{Total amount of the drug} - \text{amount of the drug detected in the supernatant}}{\text{Weight of the total drug added}} \times 100\%$$

Drug release

Release studies can be conducted at a temperature of 37°C in a controlled environment and using a suitable release material (buffer). A dialysis membrane, which has a specific molecular weight limit, is either used as a dialysis bag or attached to the end of a tube. It is then immersed in the receptor medium and stirred.[39]

The release medium is typically buffered at pH 7.4 and maintained at 37°C with stirring to simulate in vivo conditions (in vitro drug release). Samples of the medium are extracted at specific time intervals to determine the concentration using various analytical techniques such as HPLC and UV–Vis spectrophotometry. The extracted samples are then replaced with an equal volume of fresh medium to maintain a constant volume of receptor medium.[40]

CONCLUSION

The dermal and transdermal drug delivery system is an advanced method of administering drugs that provides prolonged controlled release of both hydrophobic and hydrophilic drugs. Furthermore, it has shown significant promise in efficiently solving the problem of limited oral drug bioavailability and the discomfort associated with injections.

The main challenge in transdermal drug delivery lies in the skin barrier, which hinders the penetration of most drugs. Therefore, in order to address the emerging issue, various nanocarriers and vesicle systems have been developed and modified to optimize the drug therapy process.

These vesicle and particle systems have been studied for their ease of preparation, safety, and effectiveness. Their inclusion in transdermal patches, creams, and gels would enhance the absorption of drugs through the skin.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

REFERENCES

[1] Limongi T, Susa F, Marini M, Allione M, Torre B, Pisano R, et al. Lipid-based nanovesicular drug delivery systems. Nanomaterials. 2021 Dec 14;11(12):3391. doi: 10.3390/nano11123391.

[2] Edis Z, Wang J, Waqas MK, Ijaz M, Ijaz M. Nanocarriers-mediated drug delivery systems for anticancer agents: An overview and perspectives. Int J Nanomed. 2021 Feb 17:16:1313–30. doi: 10.2147/IJN.S289443.

[3] Amoretti M, Amsler C, Bonomi G, Bouchta A, Bowe P, Carraro C, et al. Production and detection of cold antihydrogen atoms. 2002 Oct 3;419(6906):456–9. doi: 10.1038/nature01096.

[4] Kurakula M, Chen L, Tiwari AK, Srinivas NR, Dash RP, Panizzi P, et al. Recent advances in lipid-based nanovesicular delivery systems for melanoma therapy. Crit Rev Ther Drug Carrier Syst. 2021;38(4):1–38. doi: 10.1615/CritRevTherDrugCarrierSyst.2021034903.

[5] Ali Ashoor J, Mohsin JM, Mohsin HM, Mahde BW, Gareeb MM. Permeability enhancement of methotrexate transdermal gel using eucalyptus oil, peppermint oil and olive oil (Conference Paper)#. Iraqi J Pharm Sci. 2022 Jan 11;30(Suppl.):16–21. doi: 10.31351/vol30issSuppl.pp16-21.

[6] Al-Edhari GH, Al Gawhari FJ. Study the effect of formulation variables on preparation of nisoldipine loaded nano bilosomes. Iraqi J Pharm Sci. 2023 Nov. 4 [cited 2024 Sep. 21];32(Suppl.):271–82. doi: 10.31351/vol32issSuppl.pp271-282.

[7] Ali SK, Al-Akkam EJ. Bilosomes as soft nanovesicular carriers for ropinirole hydrochloride: Preparation and in- vitro characterization. Iraqi J Pharm Sci. 2023 Nov 3;32(Suppl.):177–87. doi: 10.31351/vol32issSuppl.pp177-187.

[8] Lakshmi NM, Yalavarthi PR, Vadlamudi HC, Thanniru J, Yaga G, KH. Cubosomes as targeted drug delivery systems – A biopharmaceutical approach. Curr Drug Discov Technol. 2014 Nov 28;11(3):181–8. doi: 10.2174/1570163811666140-505125923.

[9] Sivadasan D, Sultan MH, Alqahtani SS, Javed S. Cubosomes in drug delivery—A comprehensive review on its structural components, preparation techniques and therapeutic applications. Biomedicines. 2023 Apr 7;11(4):1114. doi: 10.3390/biomedicines11041114.

[10] Chacko IA, Ghate VM, Dsouza L, Lewis SA. Lipid vesicles: A versatile drug delivery platform for dermal and transdermal applications. Colloids Surf B Biointerfaces. 2020 Nov;195:111262. doi: 10.1016/j.colsurfb.2020.111262.

[11] Barriga HMG, Holme MN, Stevens MM. Cubosomes: The next generation of smart lipid nanoparticles? Angew Chem Int Ed. 2019 Mar 4;58(10):2958–78. doi: 10.1002/anie.201804067.

[12] Gaballa SA, El Garhy OH, Abdelkader H. Cubosomes: Composition, preparation, and drug delivery applications. J. Adv. Biomed. Pharm. Sci. 2020;3:1–9. doi: 10.21608/jabps.2019.16887.1057.

[13] Garg M, Goyal A, Kumari S. An update on the recent advances in cubosome: A novel drug delivery system. Curr Drug Metab. 2021;22(6):441–50. doi: 10.2174/1389200221666210105121532.

[14] Sen R, Gupta R, Singh S, Mantry S, Das S. A review on cubosome and virosome: The novel drug delivery system. UJPSR. 2017;3(1):24–33. doi: 10.21276/UJPSR.2017.03.01.99.

[15] Mansour M, Kamel AO, Mansour S, Mortada ND. Novel polyglycerol-dioleate based cubosomal dispersion with tailored physical characteristics for controlled delivery of ondansetron. Colloids Surf B Biointerfaces. 2017 Aug 1:156:44–54. doi: 10.1016/j.colsurfb.2017.04.052.

[16] Kumar P, Singh SK, Handa V, Kathuria H. Oleic acid nanovesicles of minoxidil for enhanced follicular delivery. Medicines (Basel). 2018 Sep 14;5(3):103. doi: 10.3390/medicines5030103.

[17] Bagmar NA, Hatwar PR, Bakal RL. A review on targeted drug delivery system. World J Pharm Res. 2023 Oct 19;12(19):288–98. doi: 1020959/wjpr202319-30070.

[18] Luke PM, Joseph T. Ufasomes: Rising technology for delivery of drugs. Int J Med Pharm Sci. 2021 Jan 1;11(11):1–7. doi: 10.31782/IJMPS.2021.111101.

[19] Nair AJ, Aswathi K, George A, Athira PP, Nair SC. Ufasome: A potential phospholipid carrier as a novel pharmaceutical formulation. Int Res J Pharm. 2014 Apr 19;5(4):250–3. doi: 10.7897/2230-8407.050453.

[20] Arundhasree, Rajalakshmi R, Aiswarya R, Kumar AR, Kumar SS, Nair SC. Ufasomes: Unsaturated fatty acid based vesicular drug delivery system. Int J Appl Pharm. 2021 Mar 7;13(2):76–83.doi:10.22159/ijap.2021v13i2.39526.

[21] Fareed NY, Kassab HJ. Effect of formulation variables on the properties of a new vesicular system of an anthraquinone derivative. J Adv Pharm Educ Res. 2023;13(4):25–9. doi: 10.51847/JDGOLCgVXm.

[22] Patel DM, Patel CN, Jani RH. Ufasomes: A vesicular drug delivery. Syst Rev Pharm. 2011 Jul–Dec;2(2):72–8. doi: 10.4103/0975-8453.86290.

[23] Patel H. A vesicular drug delivery for futuristic drug delivery applications: Ufasomes. Ind J Pharm Biol Res. 2022Apr;10(4):1–7.

[24] Nangare S, Dugam S. Smart invasome synthesis, characterizations, pharmaceutical applications, and pharmacokinetic perspective: A review. Futur J Pharm Sci. 2020;6:123. doi: 10.1186/s43094-020-00145-8.

[25] El-Nabarawi MA, Shamma RN, Farouk F, Nasralla SM. Dapsone-loaded invasomes as a potential treatment of acne: Preparation, characterization, and in vivo skin deposition assay. AAPS PharmSciTech. 2018 Jul 1;19(5):2174–84. doi: 10.1208/s12249-018-1025-0.

[26] Salih OS, Al-Akkam EJ. Pharmacokinetic parameters of ondansetron in rats after oral solution and transdermal invasomes gel: A comparison study. J Adv Pharm Educ Res. 2023;13(1):116–21. doi: 10.51847/HS5a27El60.

[27] Afreen U, Sailaja AK. Overall review on invasomes. Res J Nanosci Eng. 2019 Jan 1;3(4):5–9. doi: 10.22259/2637-5591.0304002.

[28] Salih OS, Al-akkam EJ. Invasomes as a novel delivery carrier for transdermal delivery: Review article. Medicon Pharm Sci. 2022 Apr; 30;2(3):11–16.

[29] Kaur L, Kaur P, Khan M. Liposome as a drug carrier – A review. IJRPC Int J Res Pharm Chem. 2013;3(1):121–8. Available from: https://www.ijrpc.com/files/20-342.pdf

[30] Li T, Nowell CJ, Cipolla D, Rades T, Boyd BJ. Direct comparison of standard transmission electron microscopy and cryogenic-TEM in imaging nanocrystals inside liposomes. Mol Pharm. 2019 Apr 1;16(4):1775–81. doi: 10.1021/acs.molpharmaceut.8b01308.

[31] Nasr M, Saber S, Bazeed AY, Ramadan HA, Ebada A, Ciorba AL, et al. Advantages of cubosomal formulation for gatifloxacin delivery in the treatment of bacterial keratitis: In vitro and in vivo approach using clinical isolate of methicillin-resistant *Staphylococcus aureus*. Materials (Basel). 2022 May 8;15(9):3374. doi: 10.3390/ma15093374.

[32] Miere (Groza) F, Fritea L, Cavalu S, Ioana Vicaş S. Formulation, characterization, and advantages of using liposomes in multiple therapies. Pharmacophore. 2020 Jan;11(3):1–12.

[33] Prasanthi D, Lakshmi PK. Iontophoretic transdermal delivery of finasteride in vesicular invasomal carriers. Pharm Nanotechnol. 2013 Apr 1;1(2):136–50. doi: 10.2174/2211738511301020009.

[34] Laouini A, Jaafar-Maalej C, Limayem-Blouza I, Sfar S, Charcosset C, Fessi H. Preparation, characterization and applications of liposomes: State of the art. J Colloid Sci Biotechnol. 2012 Dec 1;1(2):147–68. doi: 10.1166/jcsb.2012.1020.

[35] Nasr M, Almawash S, Al Saqr A, Bazeed AY, Saber S, Elagamy HI. Bioavailability and antidiabetic activity of gliclazide-loaded cubosomal nanoparticles. Pharmaceuticals (Basel). 2021 Aug 9;14(8):786. doi: 10.3390/ph14080786.

[36] Fan Y, Marioli M, Zhang K. Analytical characterization of liposomes and other lipid nanoparticles for drug delivery. J Pharm Biomed Anal. 2021 Jan 5:192:113642. doi: 10.1016/j.jpba.2020.113642.

[37] Jain AK, Thareja S. In vitro and in vivo characterization of pharmaceutical nanocarriers used for drug delivery. Artif Cells Nanomed Biotechnol. 2019 Dec;47(1):524–39. doi: 10.1080/21691401.2018.1561457.

[38] Alkawak RSY, Rajab NA. Lornoxicam-loaded cubosomes: – Preparation and in vitro characterization. Iraqi J Pharm Sci. 2022 Jun 17;31(1):144–53. doi: 10.31351/vol31iss1pp144-153.

[39] Kassab HJ Alkufi HK, Hussein LS. Use of factorial design in formulation and evaluation of intrarectal in situ gel of sumatriptan. J Adv Pharm Technol Res. 2023 Apr–Jun; 14(2):119–124. doi: 10.4103/japtr.japtr_603_22.

[40] Ahmed KS, Hussein SA, Ali AH, Korma SA, Lipeng Q, Jinghua C. Liposome: Composition, characterisation, preparation, and recent innovation in clinical applications. J Drug Target. 2019 Aug;27(7):742–761. doi: 10.1080/1061186X.2018.1527337.